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# Linking evolutionary ecology with epidemiology*

Jacob C. KOELLA
Experimental Ecology, ETH Zentrum NW,
CH-8092 Zürich, Switzerland.

**Linking evolutionary ecology with epidemiology. –** The benefit of linking evolutionary ecology with epidemiology is discussed with two examples chosen from malaria epidemiology: the spread of chloroquine resistance, and an increase of the intensity of transmission over time in north-east Tanzania. Differences in the epidemiological patterns of chloroquine resistance in different geographical areas have long been known. Considering these differences from an ecological and evolutionary viewpoint, in particular using population genetics and frequency dependent selection together with models of the transmission of malaria, suggests that they arise because chloroquine resistance is linked to the parasite's immunological properties of malaria parasites. The intensity of transmission of malaria in north-east Tanzania increased considerably in the 25 years from 1965 to 1990. A possible explanation for this is that during this time chloroquine resistance has emerged and become common, and that resistant parasites are more easily transmitted than sensitive parasites. This suggestion finds indirect support from theoretical and laboratory based work on the evolutionary ecology of parasites. The theoretical work suggests that, in many cases, the time at which transmission stages are produced should increase as replication rate of a parasite increases. This prediction is supported by *in vitro* studies on the development of the transmission stages of malaria parasites. Furthermore, because rapidly replicating parasites tend to be sensitive, it follows that resistance *in vitro* is associated with early, and thus intense, transmission.

**Key-words:** Evolutionary ecology – Epidemiology – Malaria – Chloroquine – Resistence.

## INTRODUCTION

The sciences of evolutionary ecology and epidemiology have largely progressed independent of each other. Only recently have attempts been made to merge the two schools. The most influential of these attempts has focused on the evolution of parasite

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virulence (e.g. ANDERSON & MAY, 1982; EWALD, 1983), undermining the conventional wisdom that all parasites should evolve towards avirulence. Tbeory and data make it clear that natural selection can favour parasites with intermediate virulence or can lead to ever-increasing virulence. Most other work deals with the evolution of hosts in response to parasite pressure. Epidemiological models of host-parasite systems suggest, for example, that sexual reproduction is maintained by the pressure to continually evolve away from the rapidly evolving parasites (HAMILTON, 1980; HAMILTON & *al.*, 1990; LADLE, 1992) and that parasites are responsible for the evolution of preference for mating partners bearing extravagant traits (HAMILTON & ZUK, 1982).

Thus, there has been some diffusion of epidemiology into evolutionary ecology. The integration of evolutionary and ecological ideas into epidemiology, however, is largely absent. Such an integration could contribute to a better understanding of epidemiological patterns and distribution of disease, in particular because, in contrast to more traditional epidemiological thinking, evolutionary ecology emphasises the continually changing parameters expected in a host-parasite system. I illustrate the benefit of linking evolutionary ecology and epidemiology with two examples chosen from malaria: the spread of chloroquine resistance, and the changes of the intensity of transmission over time. Both of these examples emphasise the importance of the evolution of the parasite and of its dynamics within its host for an understanding of its epidemiology.

## SPREAD OF CHLOROQUINE RESISTANCE

The emergence of chloroquine resistant genotypes of the malaria parasite *Plasmodium falciparum* is one of the major factors that has prevented global eradication of malaria. Although much effort has been put into understanding the molecular mechanisms of chloroquine resistance, many questions about its distribution remain unanswered. Why, for example, has chloroquine resistance, despite extensive use of the drug, not yet become fixed some 30 years after its emergence, but have many parasites remained sensitive to the drug? Why, after its first appearance in Africa, has chloroquine resistance spread so rapidly through sub-Saharan Africa? A possible answer is that a parasite's resistance is associated with its other traits. Indeed, an association has been suggested between resistance and the parasite's immunological properties (PETERS, 1987; KOELLA & *al.*, 1990; KOELLA, 1993). Such an association leads to frequency-dependent selection if, as is generally accepted (e.g. FORSYTH & *al.*, 1989; MENDIS & *al.*, 1991), immunity against malaria is genotype specific. Therefore, as the frequency of parasites that are resistant against chloroquine increases, the level of immunity in the human population against these parasites increases, so that the advantage of being resistant decreases. Thus, different immunological properties of resistant and sensitive parasites would explain why resistance initially spreads very rapidly in areas with intense transmission and extensive use of chloroquine, but subsequently remains fairly stable.

An association between drug resistance and immunological properties will lead to an evolutionary pattern similar to that in Figure 1. This pattern is based on the fact that protective immunity against malaria develops very slowly, which means that very young children are susceptible to disease, whereas older children and adults are essentially protected. During the early phases of the spread of resistance, the most common parasites are sensitive, and it is against these that immunity develops. The parasites surviving the immune system of people that are old enough to have developed protective immunity will thus be on average more resistant than the parasites obtained from non-immunes. On the other hand, when resistance has reached an equilibrium and most parasites are resistant, immunity against resistant parasites will predominate, so that the parasites obtained from immunes will be mostly sensitive. These dynamics lead

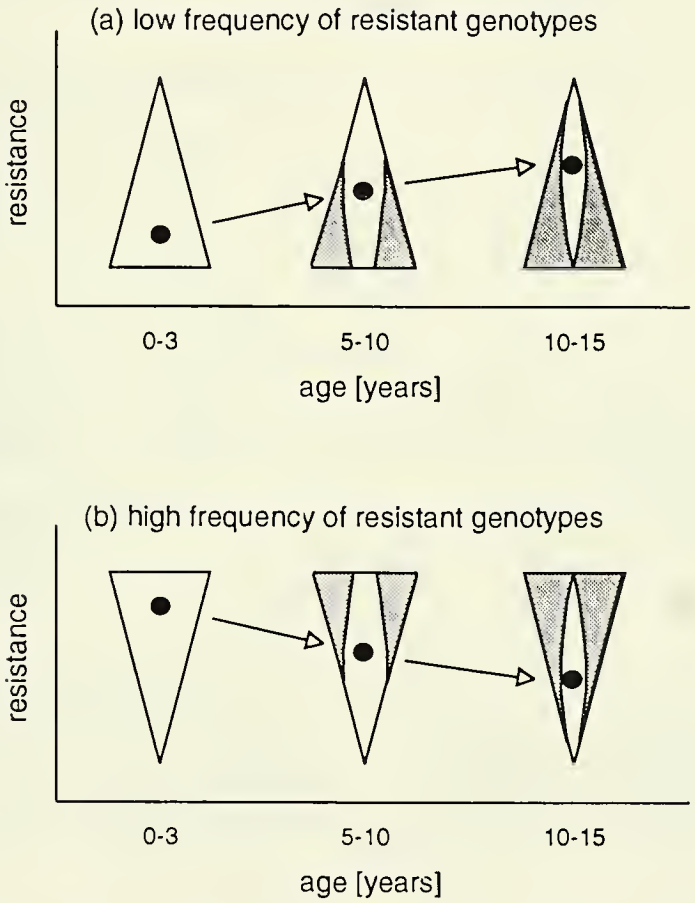


FIG. 1

Schematic presentation of the effect of immunological differences between resistant and sensitive parasites on the evolution of resistance. The resistance of parasites isolated from children at different ages, and thus with different levels of protective immunity, is sketched for areas with (a) low and (b) high frequencies of resistant parasites. The relative frequencies of individual isolates that infect children are indicated by the width of the triangles. The parasites cleared by the immune system are shown as the stippled area. The mean resistance of the parasites that survive the immune system, and can thus be isolated, is shown as a solid circle.

to three testable predictions. First, when resistance has reached equilibrium, it should decrease with the age of the person from whom the parasites were obtained. Second, shortly after the emergence of resistance, we should observe a negative interaction between time since emergence and the age of the person from whom the parasites were obtained. Third, in areas with little transmission, i.e. where no immunity is developed, resistance should not depend on the age of the person.

Support for an association between resistance and immunological properties was first observed in Ifakara, Tanzania (KOELLA & *al.*, 1990), where resistance first appeared in 1981. Between 1982 and 1988 resistance of parasites obtained from children less than three years old increased steadily, whereas the resistance of parasites obtained from 12-15 year old school children decreased from very high levels in 1986 to that from younger children in 1989 (Fig. 2). This is consistent with the second prediction, a negative interaction between time since emergence and the age of the children from whom the parasites were obtained.

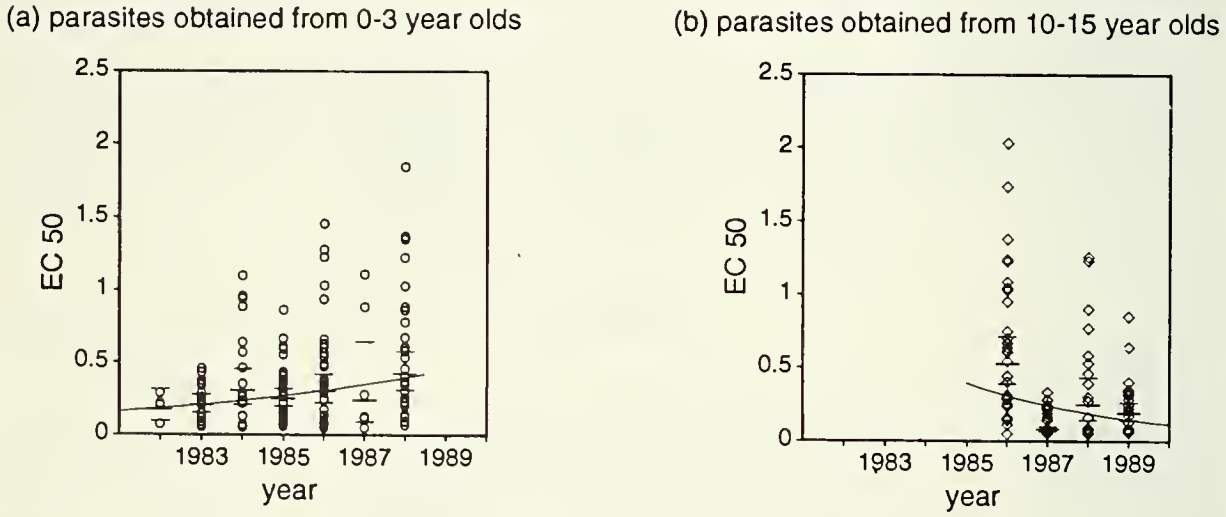


FIG. 2

The resistance of each isolate obtained from under three year old children (a) and from ten to fifteen year old schoolchildren (b). Resistance is measured as $EC_{50}$, i.e. the concentration of chloroquine that reduces the replication rate of the parasite to 50% of the control value. High values of $EC_{50}$ are thus associated with high resistance. The curves show the regressions of $\log(EC_{50})$ against year. The slopes of the curves were 0.126 ($p<0.001$) for the under three year old and -0.233 ($p<0.001$) for the schoolchildren.

Further evidence is obtained from WHO's database on drug resistance, which contains the results of all reported resistance tests. The analysis shown used the resistance for samples obtained between 1981 and 1989 from children less than 15 years old. The data were grouped into geographical areas containing countries with similar intensities of transmission (Table 1). For each area, an analysis of variance (Table 2) tested whether resistance of parasites was associated with time since emergence of resistance, age of the child from whom the parasites were obtained or the interaction of the two. Its results were consistent with the three predictions. In areas with low transmission age had no effect on resistance. In areas with more intense transmission and

TABLE 1

Geographical areas of differing intensity of transmission of malaria. Only those countries are listed that have sufficient data for the analysis.

| Low transmission | Intermediate transmission | | Intense transmission | |
|---|---|---|---|---|
| West Asia | Southeast Asia | Southeast Asian Islands | East Africa | West Africa |
| Afghanistan | Bangladesh | Indonesia | Burundi | Angola |
| Iran | Laos | Philippines | Kenya | Cameroun |
| Nepal | Malaysia | Vanuatu | Madagascar | Central African Republic |
| Oman | Thailand | | Malawi | Côte d'Ivoire |
| Pakistan | Vietnam | | Mozambique | Gabon |
| Saudi Arabia | | | Tanzania | The Gambia |
| Yemen | | | Uganda | Guinea |
| | | | Zambia | Guinea-Bassau |
| | | | | Liberia |
| | | | | Nigeria |
| | | | | Zaire |

TABLE 2

Analysis of variance of the association of resistance and year since emergence of resistance and age of the child from whom the parasites were isolated. The interaction term is shown only where it was statistically significant at $p<0.05$. Resistance is given as $EC_{50}$, i.e. the concentration of chloroquine that reduces the replication rate of the parasite to 50% of the control value.

| **Source** | df | SS (Type III) | F value | p | Estimate |
|---|---|---|---|---|---|
| **East Asia** | | | | | |
| year | 1 | 0.307 | 0.38 | 0.54 | –0.024 |
| age | 1 | 1.287 | 1.60 | 0.20 | –0.024 |
| year * age | – | – | – | ns | |
| **Asian Islands** | | | | | |
| year | 1 | 11.866 | 12.70 | <0.001 | 0.267 |
| age | 1 | 6.432 | 6.88 | 0.009 | 2.351 |
| year * age | 1 | 6.255 | 6.70 | 0.01 | –0.028 |
| **East Africa** | | | | | |
| year | 1 | 42.272 | 43.69 | < 0.001 | 0.314 |
| age | 1 | 28.252 | 29.20 | < 0.001 | 2.671 |
| year * age | 1 | 28.211 | 29.16 | < 0.001 | –0.031 |
| **West Africa** | | | | | |
| year | 1 | 22.211 | 35.72 | < 0.001 | 0.279 |
| age | 1 | 5.573 | 8.96 | 0.003 | 2.076 |
| year * age | 1 | 5.741 | 9.23 | 0.002 | –0.025 |
| **West Asia** | | | | | |
| year | 1 | 2.546 | 5.95 | 0.02 | –0.073 |
| age | 1 | 0.228 | 0.53 | 0.47 | –0.013 |
| year * age | – | – | – | ns | |

where resistance was increasing (i.e. where resistance had not yet reached its equilibrium), resistance initially increased and later decreased with age. In areas with intense transmission and where resistance remained constant, no interaction between age and year was observed.

Thus, epidemiological data suggest that chloroquine resistance and immunological properties of malaria parasites are associated. This conclusion was reached with a combination of concepts from epidemiology (knowledge of the distribution of disease), ecology (dynamics of the host-parasite interaction) and evolutionary theory (population genetics leading to frequency dependent selection and trade-offs).

## PARASITE LIFE CYCLES AND EPIDEMIOLOGY

The intensity of transmission of malaria in north-east Tanzania increased considerably in the 25 years from 1965 to 1990 (Lines & *al.*, 1991). Lines & *al.* eliminated several factors, including climatic changes, as causes, and suggested instead that the increase in intensity of transmission is due to the extensive use of chloroquine. However, evidence is lacking.

Some indirect support can be obtained from quantitative studies on the dynamics of the malaria parasite's life cycle Malaria produces two stages within its human host: an asexual stage that replicates extensively within its host, and a sexual stage that cannot replicate but is taken up by a mosquito vector and allows transmission of malaria to another host individual. A typical example of the dynamics of these two stages is shown in Fig. 3.

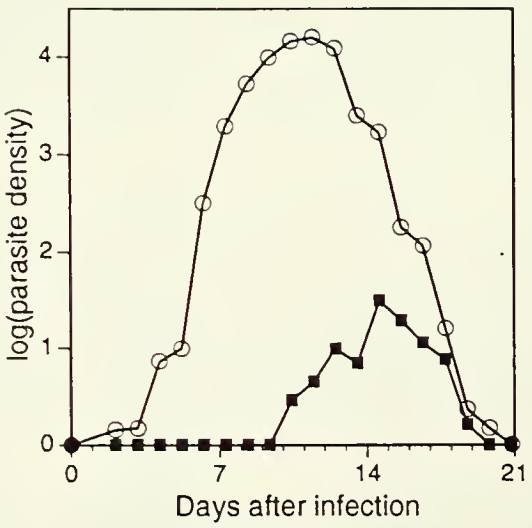


Fig. 3

The mean dynamics of *Plasmodium chabaudi chabaudi* within inbred mice during the initial period of infection. Each mouse was infected with a total of 5x104 parasites on day 0. The open circles show the geometric mean density of replication stages, the closed squares the geometric mean density of transmission stages measured in the blood of the mouse. Both densities are shown as the logarithm of the number of parasites per $10^5$ erythrocytes. The later period of the infection, in which recrudescence up to low densities of both stages of the parasite occur, is

The dynamics of parasites such as malaria, with two stages within their host, were modelled by KOELLA & ANTIA (1994). The model describes the replication of the asexual stages, their conversion to transmission stages, and the interaction of the asexual stages with the host's immune system. It was used to calculate the pattern of investment into transmission stages that maximises the parasite's potential for transmission. The predicted pattern of investment largely depends on the replication rate of the parasite and the rate of development of the immune system. If the immune system can clear the parasite before it reaches a density at which it would kill the host, the optimal investment follows a bang-bang pattern; the parasite switches from no production of transmission stages to complete investment at a time shortly before it is cleared. If the parasite does reach lethal density, it achieves maximal transmission, if it replicates as rapidly as possible up to lethal density, i.e. if initially it does not invest into transmission. It then increases its investment to a level that reduces its net rate of replication to zero, so that the density of the asexual stages remains just below the lethal density. Finally, shortly before it is cleared by the immune system, it switches to complete investment into transmission. From this optimal pattern of investment into transmission it follows that parasites that replicate rapidly and can kill their hosts at low densities should produce transmission stages earlier than their more slowly growing, avirulent counterparts. Qualitative data on 16 *Plasmodium* species (obtained from GARNHAM, 1966) lend some support to the idea (Table 3), although the data are insufficient to test the prediction quantitatively.

TABLE 3

The time of the first appearance of transmission stages of various species of *Plasmodium*. The species are grouped by the rate of replication of the parasites and by the degree of virulence. Parasites with rapid replication have a doubling time of less than 12 hours, slow parasites more than 12 hours. Highly virulent parasites often kill their hosts, intermediately virulent parasites rarely kill their host but cause noticeable disease, avirulent parasites have only little noticeable effect on their host. The time of appearance is given within the parentheses as the day after the first replication stages are observed in the blood of the host. The data are taken from GARNHAM (1966).

| Rapid replication | Slow replication | Slow replication |
|---|---|---|
| High virulence | Intermediate virulence | Low virulence |
| *P. berghei* [1] (1-2) | *P. chabaudi* [1] (6) | *P. brasilianum* (14-21) |
| *P. knowlesi* (3) | *P. falciparum* [2] (2-5) | *P. cynomolgi* (9) |
| *P. simiovale* (2) | *P. fragile* (3-4) | *P. gonderi* (14) |
| *P. vinckei* [1] (1-2) | *P. vivax* (0-5) | *P. inui* (14) |
| | | *P. malariae* (10-23) |
| | | *P. ovale* (5) |
| | | *P. shortii* (7) |

1: Rodent malaria; all other species infect primates.
2: Time of appearance of initial form of the transmission stages. The mature form appears some ten days later.

A more detailed analysis of the model leads to the prediction that, if a parasite generally does not kill its host, the time it switches to transmission should be positively correlated with the replication rate of its asexual stages. This prediction was supported for *Plasmodium falciparum* by HUBER's (1991) study on its *in vitro* dynamics (Fig. 4a). The association between the dynamics of the asexual and the investment into sexual stages seems to be linked to a cost of resistance in that isolates containing resistant parasites replicated less rapidly than did sensitive isolates (Fig. 4b). On the other hand

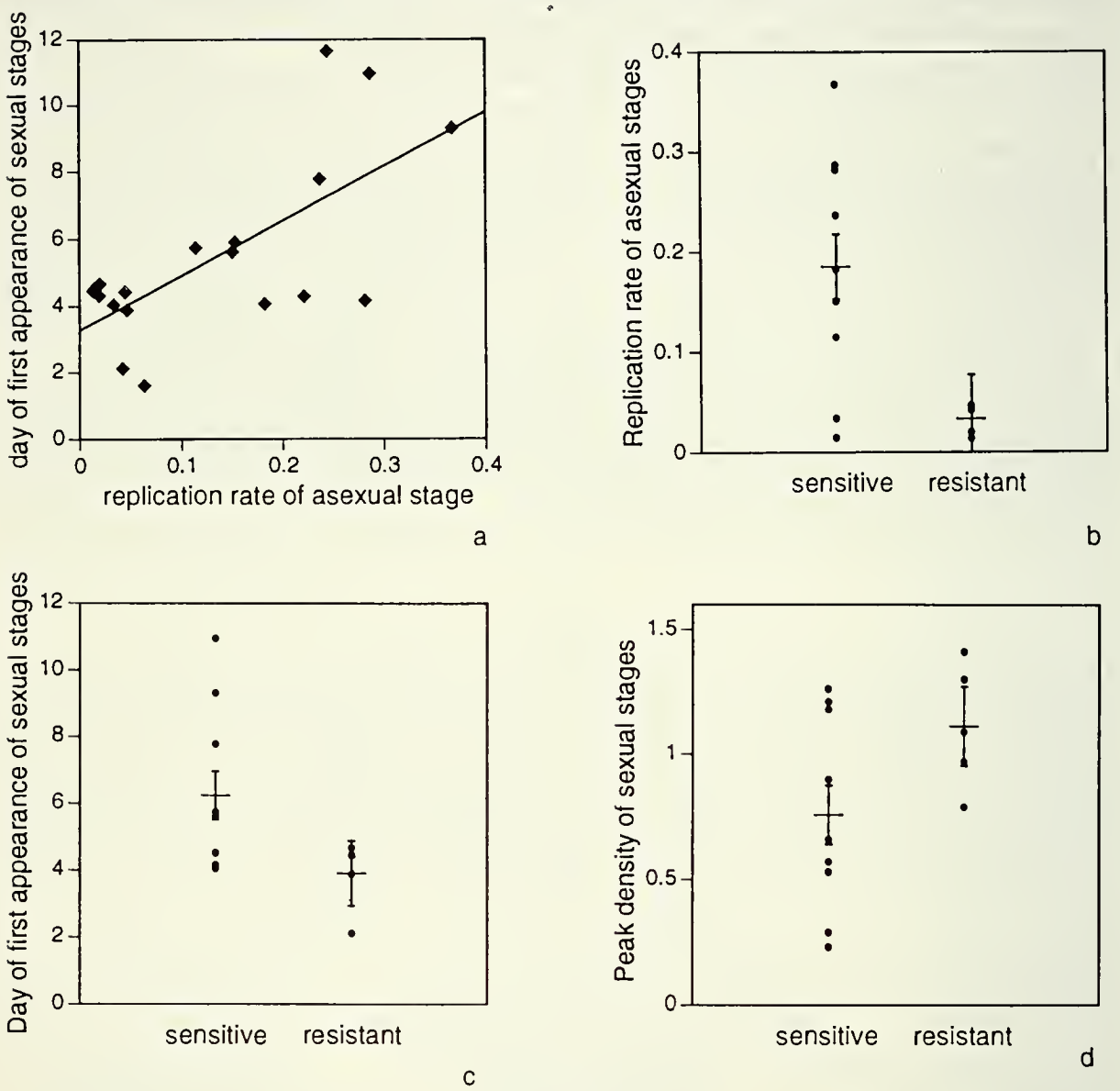


FIG. 4

Results of studies on the *in vitro* dynamics of *Plasmodium falciparum* (HUBER, 1991). (a) Association between replication rate of the asexual stages and time of emergence of the first sexual stages. (b) Association between resistance and replication rate. (c) Association between resistance and time of emergence of sexual stages. (d) Association between resistance and peak density of sexual stages. In each graph, the dots show individual isolates, the horizontal bars show the means and the vertical bars show the standard errors of the mean. Resistance is given as $EC_{50}$, i.e. the concentration of chloroquine that reduces the replication rate of the parasite to 50% of the control value.

the dynamics of the production of transmission stages were also affected by resistance; transmission stages of resistant parasites were observed earlier (Fig. 4c) and at a higher density (Fig. 4d) than were those of sensitive parasites. If this pattern holds true *in vivo*, it would suggest a higher potential for transmission of resistant than of sensitive parasites. Thus, the increase of transmission intensity in the past 30 years may be due to the evolution and spread of chloroquine resistance.

If these suggestions were true, they would describe an interpretation of epidemiological data that would be difficult to find or test without explicity investigating the evolution of the malaria life cycle. Note that all of the evolutionary concepts described here are parts of a standard toolbox used by many evolutionary biologists; cost of resistance and life-history theory. Thus, what are standard tools in evolutionary ecology can help to explain patterns in epidemiology.

## DISCUSSION

This paper emphasises that epidemiology can profit from evolutionary ideas, more explicitly that evolutionary ideas can help to explain patterns of disease. The suggestions, furthermore, demonstrate that using available knowledge to make predictions about evolutionary changes would help to make disease control programs more efficient. It seems likely that extensive use of chloroquine will render the drug useless in a short time, because resistance will evolve and spread rapidly. A more detailed view suggests on the one hand that resistance may not be fixed because of frequency dependent selection. On the other hand it suggests that extensive use of chloroquine may not just make the drug more or less useless, but that it might lead to the opposite of what one wishes to achieve: a higher intensity of transmission.

Note that the examples also show how parasitology and epidemiology will lead evolutionary ecologists to new ideas about old problems. Parasites, for examples, can be used to test ideas about selection operating at several levels: replication within individual hosts, and transmission among hosts. Finally, both examples touch on a newly developing field, which considers the interactions of parasites and the immune system. It describes the selection pressure imposed onto the parasite within its host by the immune system and how this selection pressure can determine the parasite's virulence or life cycle.

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